EFFECT OF SILVER AND RESIN REINFORCED GLASS IONOMERS ON THE ENAMEL MICROHARDNESS AFTER EXPOSURE TO CARIOGENIC BIOFILM

Beatriz Portela Teixeira Da **Silva**[1], Cynthia **Carvalho**[2], Viviane **Cancio**[2], Fernanda **Calazans**[1], Antônio **Ferreira-Pereira**3, Maristela Barbosa **Portela**[2]*, Monica Almeida **Tostes**[2]

[1]Department of Restorative Dentistry, Universidade Federal Fluminense – UFF, Nova Friburgo, Rio de Janeiro, Brazil.
[2]Department of Dental Clinic, Faculty of Dentistry, Universidade Federal Fluminense - UFF, Nova Friburgo, Rio de Janeiro, Brazil.
[3]Department of General Microbiology, Universidade Federal do Rio de Janeiro – UFRJ, Rio de Janeiro, Rio de Janeiro, Brazil.

Palavras-chave: Testes de Dureza. Resinas Compostas. Esmalte Dentário. Cimentos de Ionômeros de Vidro.

RESUMO

Objetivo: Comparar a desmineralização nas margens da interface dente/restauração utilizando cimento de ionômero de vidro modificado com resina (RI) e reforçado com prata (RS) e com resina composta (CO) após desafio cariogênico. **Materiais e Métodos**: 30 blocos de esmalte bovino com cavidades padronizadas foram divididos em 3 grupos de acordo com os materiais utilizados: RI (Riva Light Cure™, SDI), RS (Riva Silver™, SDI) e CO (Filtek™ Z350 XT, 3M). Metade de cada superfície de esmalte restaurada foi protegida com verniz ácido-resistente. Os blocos foram expostos ao biofilme de Streptococcus mutans. O verniz foi removido dos espécimes com algodão e álcool para mensuração da microdureza superficial (MDS - Knoop, 50 g, 15 s), através de 3 linhas com 5 indentações em cada e 100 µm de distância entre elas. Os dados foram submetidos ao programa SPSS 20.0, teste de normalidade de Shapiro Wilk, Kruskal Wallis e Mann Whitney ($p<0,05$). **Resultados**: A análise da MDS demonstrou que na distância de 50 µm da restauração, o grupo RS apresentou ganho percentual de dureza (6,31 ± 0,01), diferentemente dos grupo RI (-0,036 ± 0,05) e CO (-11,43 ± 0,02) que apresentaram perda significativa ($p<0,05$). Nas demais distâncias, não foi observada diferença estatística entre os grupos. **Conclusão**: Todos os cimentos de ionômero de vidro aumentaram a microdureza superficial total dos blocos de esmalte mesmo após exposição ao biofilme cariogênico. No entanto, apenas o grupo RS impediu a desmineralização a 50 µm das margens de restaurações submetidas a biofilme cariogênico.

Keywords: Dental Enamel. Composite Resins. Glass iIonomer Cements. Hardness Tests.

ABSTRACT

Objective: To assess the superficial microhardness of enamel-restorations margins of glass ionomer cement reinforced with silver (RS), modified with resin (RI) and composite resin (CO) after cariogenic biofilm. **Materials and Methods**: Thirty bovine enamel blocks with standard cavities were divided into three groups according to the materials used: RI (Riva Light Cure™, SDI), RS (Riva Silver™, SDI) and CO (Filtek™ Z350 XT, 3M). Half of each enamel block surface was covered by acid resistant varnish. After that, the blocks were exposed to Streptococcus mutans biofilm. The varnish was removed from the blocks and superficial microhardness (MDS) was measured (Knoop, 50 g, 15 s), with five indentations, 100 µm from each other in three different directions. The data were analyzed by the Shapiro Wilk, Kruskal Wallis and Mann Whitney tests ($p<0.05$). **Results**: MDS analysis indicated that in 50 µm distance from the restoration, RS group obtained hardness gain (6.31±0.01), unlike RI (-0.36±0.05) and CO (-11.43±0.02) groups that demonstrated significant loss ($p<0.05$). In other distances did not observe statistical difference between the groups. Regardless of the distance up to 450 µm, significant high total mineral gain was observed for RS group compared to the CO group; however, RS and RI presented similar enamel microhardness. **Conclusion**: All glass ionomers increased microhardness of enamel blocks even in contact with cariogenic biofilm. Although only the silver reinforced glass ionomer prevented demineralization at the margin restorations in 50 µm from the margin.

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***Correspondence to:**
Maristela Barbosa Portela
Address: Rua Beberibe, 273/201, Ricardo de Albuquerque, Rio de Janeiro, RJ, Brazil. CEP: 21640-070
Telephone number: +55 (21) 9 7013-0568
E-mail: mbportela@id.uff.br

INTRODUCTION

Dental caries is a disease that affects both adults and children.[1,2] It is defined as the chemical dissolution of hard dental tissues by bacterial acids, products of the degradation of sugars or salivary disorder.[3] Nowadays, dentistry has acquired a focus on prevention and health promotion causing people to obtain a gain in the quality of oral health.[4] For the restoration of satisfactory conditions of the oral cavity, the stage of diagnosis is essential because it will enable a better treatment to be carried out, obtaining a better functional performance of the restoration.[5] The determining factors for the appearance of a cavity should also be evaluated, such as diet, salivary factors and exposure to fluorides and even socioeconomic factors, to prevent its recurrence.[3,6]

There are a large amount of materials and ways of treatment that can be used in restoratives procedures, and some conditions will guide you in your choice, such as the dental and patient condition.[7] Composite resins are widely used, however, in many cases it has been replaced by other materials due to its deficiencies, such as contraction of the material, lack of anti-cariogenic properties and technique sensitivity.[8] Unlike the glass ionomer cement (GIC), that has a contraction during setting, but proportional to dentin, it presents a good adhesion to dental structures, fluoride release and biocompatibility.[9] However, their resistance is flawed and to improve this, resin-modified and metal-reinforced GICs were elaborated.[10]

Although there are many studies in the literature on the performance of restorative materials in the face of a cariogenic challenge, no research has been found involving glass ionomer cement reinforced with silver and comparing its performance with the others. Therefore, the aim of the present study was to evaluate the demineralization around the GIC modified with resin and reinforced with silver restorations and to compare it with composite resin restorations after exposure to the cariogenic biofilm. The hypotheses tested were: (1) glass ionomer restorations have a lower degree of demineralization on their margins in the face of a cariogenic challenge; (2) among glass ionomer restorations, those made with silver-reinforced GIC show the lowest degree of demineralization on its margins in the face of a cariogenic challenge.

MATERIAL AND METHODS

Enamel blocks and specimen preparation

Sample size was calculated by establishing a statistical power of 0.95 and significance of 0.05 with effect size 1. At least 10 specimens were necessary for each group. Thirty-five bovine incisors teeth were cleaned and disinfected by maintaining them in an aqueous solution of 0.1% thymol for 7 days. After this time, they were kept in distilled water until needed. Figure 1 is the flow chart of the present research work.

After polishing with Robinson brush and mixture of fine pumice stone and distilled water in low rotation (Kavo, Brazil S.A.) the teeth were analyzed and chosen obeying the following criteria: absence of cracks or macroscopic defects or any other enamel alteration. One sample was cut from each crown using an ISOMET low-speed saw (Buehler, Lake Bluff, IL, USA) and one diamond disc (Buehler, Lake Bluff, IL, USA), which was separated by 4-mm diameter space, obtained enamel blocks with 4 mm X 4 mm X 2 mm. The enamel surface was ground flat with sandpaper discs (600, 1000, 1200, 2400 and 4000 grades of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA) and polished with felt paper soaked in diamond slurry (1 µm; Buehler) until a glassy-looking surface was obtained.

The microhardness of the specimens was evaluated through five indentations in random areas at the enamel surface (Knoop hardness diamond, 50 g, 15 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan). Only enamel specimens with hardness values varying up to 10% of the microhardness average of the measured blocks were selected.

After enamel block selection (n=-30), standardized cavities (1.5 mm diameter for 1.5 mm deep) were prepared at the center of the enamel surface using a flat top diamond bur with stop # 2292 (KG Sorensen, Barueri, SP, Brazil). Specimens were randomly distributed into three groups (n=10) in according with restorative materials: Group RI: GIC resin-modified (Riva Light Cure™ / SDI, Australia); Group RS: GIC metal-reinforced (Riva Silver™ / SDI, Australia); Group CO: Composite resin (Filtek™ Z350 XT, 3M).

In the composite resin group (CO), the cavities were etched with 37% phosphoric acid (3M, St Paul, MN, USA) for 15 s, rinsed off for 15 s, and blot-dried. The adhesive system (Adper Single Bond 2, 3M, St Paul, MN, USA) was applied and blot-dried by a jet of air, and photocured for 10 s following the manufacturer's instructions. In the groups of GIC's (RI and RS), the cavities were etched with Riva Conditioner (10% polyacrylic acid) for 10 s, rinsed off for 20 s, and blot-dried. Then, restoratives cements based on resin-modified (Riva Light Cure™ / SDI, Australia) and on metal-reinforced (Riva Silver™ / SDI, Australia) glass ionomer were inserted followed by the surface sealant (Riva Coat / SDI, Australia). For light-cured materials, polymerization was through the polyester strip for 20 s using light-curing unit (Radii CALL, SDI) with an irradiance of 800 mW/cm^2.

After 7 days of storage at 4%C at a relative humidity of 100%, to obtain reference surfaces for lesion depth

determination, the restoration surfaces were ground flat with sandpaper discs (5000 grades of Al_2O_3 paper; Buehler, Lake Bluff, IL, USA) and polished with felt paper soaked in diamond slurry (1 µm; Buehler). After this, the enamel surface microhardness (Knoop hardness diamond, 50 g, 15 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan) of the specimens was revaluated through five indentations in random restoration margin (distance of 50 µm from the restorative margin). Only enamel specimens with hardness values varying up to 10% of the microhardness average of the measured blocks were selected. Thereafter, half of the restoration and enamel margins were covered by two layers of nail varnish for maintenance of a sound surface reference.[11]

All the sample preparation, such as obtaining the enamel blocks, standardizing the initial surface hardness and cavity preparations, restorations with the tested dental materials, final post-restoration polishing, and protection with acid-resistant varnish on half of the tested surface was performed by the same experienced and trained examiner (examiner 1).

Biofilm formation

The biofilm was composed of *Streptococcus mutans* ATCC 25175 (American type Culture Collection, Rio de Janeiro, RJ, Brazil) for its cariogenic effect and for causing caries disease. For the disinfection of the blocks, they were exposed to ultraviolet light in the laminar flow hood for 30 min to each side, followed by placing and fixed cell culture plates in the wells.[12]

The *S. mutans* were grown in 20 ml of heart and brain infusion culture medium – BHI (Brain Heart Infusion, Difco, Sparks, EUA) supplemented with 2% sucrose at 37 °C under anaerobic conditions for 24 h. The bacterial suspension was adjusted to an optical density of 0.5 in accordance with McFarland scale (Biomérieux Brazil AS, RJ, Brazil) at 550 nm using UV/VIS spectrophotometer (Beckman Coulter DU 530, LifeScience, San Diego, CA, USA). The suspension was diluted 1:100 and 10 µl of this suspension was added to each well of the culture plates, containing a specimen with 2 ml of BHI broth supplemented with 2% sucrose. The plates were incubated in 37 °C for 5 days under microaerophilic conditions. The culture medium was renewed every 24 h of incubation.

Knoop microhardness analysis

After the biofilm formation period, another trained examiner (examiner 2) performed the Knoop microhardness analysis at the margins of the restorations on both the protected and biofilm exposed surfaces. Examiner 2 was unaware of the types of materials used in the restorations performed. For this, the samples were removed from the plates and the nail varnish was removed from the reference surface with acetone-soaked cotton wool.

The enamel surface microhardness (distance of 50 µm from the restoration margin) was measured (Knoop indentation at 50 g, 15 s with five indentations, 100 µm from each other). Three different measurements were made at each distance from the restoration margin, both for the enamel surface protected by the varnish (SMH) and for the one exposed to the cariogenic biofilm (SMH1). The percentage of hardness change for protected enamel and biofilm exposed enamel was calculated as follows: %hardness = 100(SMH1 “ SMH)/SMH.

Statistical Analysis

The data were analyzed using SPSS 2.0 version 20.0 for Windows (IBM Corporation, New York, NY, USA). The Shapiro Wilk test was first applied to verify whether data followed a normal distribution. The Kruskal–Wallis and Mann–Whitney test were carried out to compare the microhardness values according to the groups and distances between the enamel-restoration margin. The analyses were conducted at a significance level of *p*-d”-0.05.

RESULTS

After the cariogenic challenge, changes in surface microhardness were observed on the enamel/material margin in the areas exposed to *S. mutans* biofilm in all groups evaluated. However, the only significant effect was in the RS group in a 50 µm distance from the margin with higher Knoop microhardness value demonstrating resistance to demineralization. The other restorative materials at all distances, despite their changes did not present any statistically significant higher or loss in microhardness values.

At 50 µm from the margin, the other groups (RI and CO) presented loss in their microhardness results, even if it is not statistically relevant, the numbers show a lack of ability to remineralize the enamel in the face of exposure of cariogenic biofilm.

The microhardness analysis of the specimens was made through the Kruskal-Wallis tests (*p*->-0.05) did not detecting differences in all groups at all indentation’s distances. The comparison of surface microhardness at different distance intervals in each group is depicted in Table j.

The Mann-Whitney test was used to detect differences in total mineral change among the groups, regardless of the distance from the restoration. Overall, significant high mineral gain was observed for RS group compared to the CO group; however, RS and RI presented similar mineral gain on enamel adjacent to restorative materials after cariogenic biofilm (Figure 2).

***Table 1**: Percentage of hardness change in enamel adjacent to restorative materials after biofilm formation at different distances.*

	Hardness change %		
Distance	**RS**	**CO**	**RI**
50 µm	$6.31^{A}(\pm 0.01)$	$-11.43^{B}(\pm 0.05)$	$-0.36^{B}(0.02)$
150 µm	$0.97^{A}(\pm 0.01)$	$-11.31^{A}(\pm 0.07)$	$0.56^{A}(\pm 0.01)$
250 µm	$4.29^{A}(\pm 0.04)$	$-10.04^{A}(\pm 0.02)$	$3.45^{A}(\pm 0.02)$
350 µm	$2.64^{A}(\pm 0.01)$	$-9.54^{A}(\pm 0.03)$	$0.03^{A}(\pm 0.01)$
450 µm	$5.77^{A}(\pm 0.03)$	$-7.61^{A}(\pm 0.01)$	$0.45^{A}(\pm 0.03)$

Note: The values represent the averages of hardness change % in the groups at the different evaluated distances. In rows, different letters indicate statistically significant differences (p<0.05) in the groups at the same distance. In columns, the mean values did not differ statistically. RS = Glass ionomer cement metal-reinforced; CO = Composite resin; RI = Glass ionomer cement resin-modified.

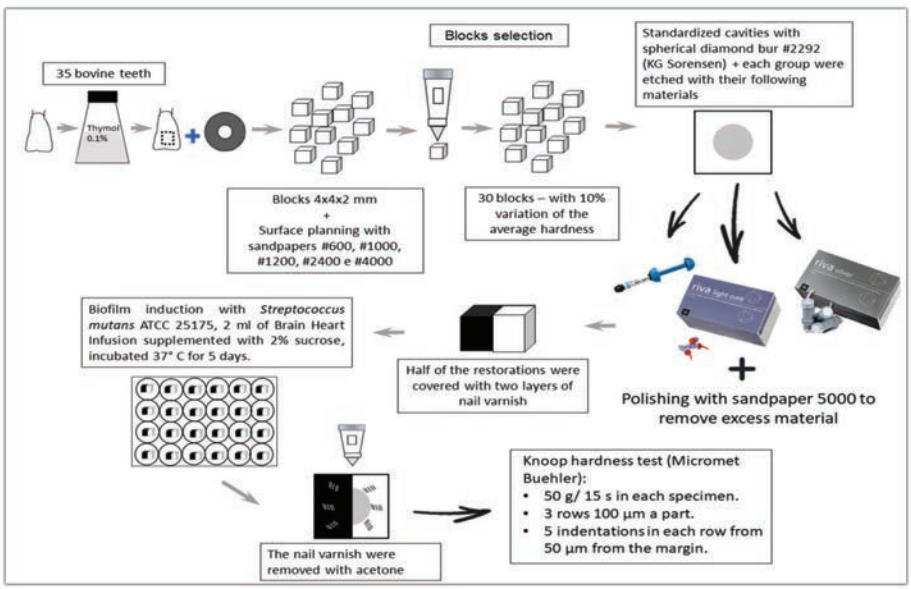

***Figure 1**: Flow chart of the research work.*

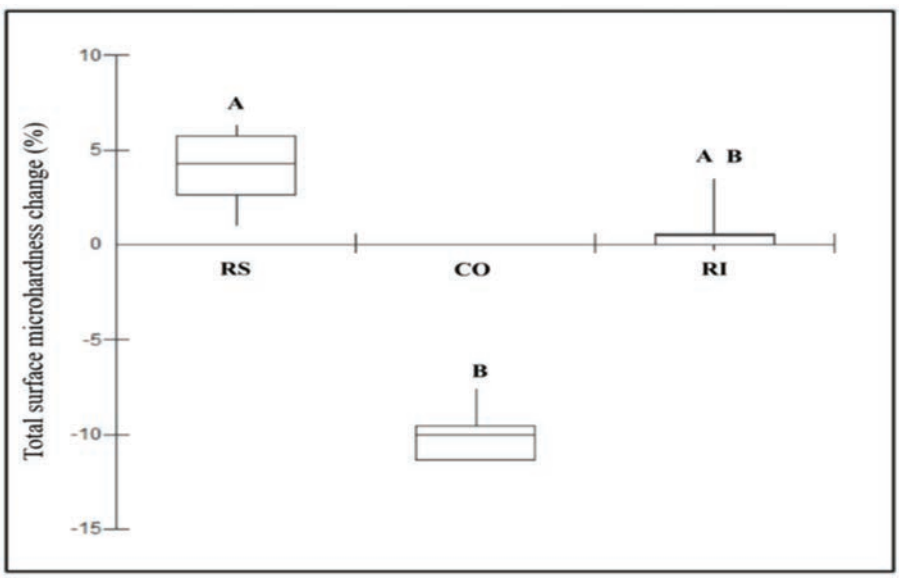

***Figure 2:** Percentage of total surface microhardness change in enamel adjacent to restorative materials after cariogenic biofilm, regardless of the distance from the restoration (median and coefficient of variation). Different letters indicate statistically significant differences (p<0.05; Mann-Whitney test). RS = Glass ionomer cement metal-reinforced; CO = Composite resin; RI = Glass ionomer cement resin-modified.*

DISCUSSION

The restorations material used in this study was restoratives cements based on resin-modified and on metal-reinforced. This material is extensively used in dentistry for both temporary and permanent restorations. Secondary or recurrent caries is a carious lesion around the margins of pre-existing restoration, most commonly in the cervical margins of restorations that are areas of biofilm accumulation.[13,14] These recurrent lesions are characterized as one of the highest reasons for the need to replace the restoration,[15] which makes its containment of great importance. So, materials with antibacterial properties are an alternative strategy to prevent secondary caries.[8]

The present in vitro study evaluated the influence of two fluoride-releasing glass ionomers for the prevention of enamel demineralization by *S. mutans* biofilm and confirmed the tested hypotheses, that the resin reinforced glass ionomer and the silver-reinforced were the least demineralized materials on the margins. The results showed that both types of glass ionomer were similar, but the inhibit demineralization effect of the RS was higher, and the difference was statistically significant. The results of the present study indicated that fluoride added to the silver particles have a strong effect in protecting the enamel demineralization. These findings should not be surprising, since that the silver nanoparticle-added sealant reduced tooth demineralization significantly and likely increased remineralization in a study *in vitro*.[16] The silver particles have been incorporated into resin, adhesive, and ionomer, showing an antimicrobial effect by inhibiting the adhesion and growth of *S. mutans*.[17] This fluoride release by the material will interfere in the metabolic activity of the biofilm and will inhibit demineralization and initiate remineralization on the surface adjacent to the enamel and at the tooth / restoration interface.[18] This release starts with a large amount of fluoride, which is subsequently reduced, however it remains constant for years, and can be influenced by several factors such as temperature, the contact surface, the storage method, and the proportion of powder and liquid of the material.[19-21] These findings suggest an association between the level of fluoride and silver particles in biofilm and anti-caries efficacy in dental products.

Some in vitro studies have confirmed the caries protect potential of GIC, due to its ability to release fluoride.[22,24] This resistance to cariogenic attacks at the enamel-/ restoration interface is of great importance in preventing secondary caries.[25] However, in the present study the RI group did not present significant values in relation to the CO group. This result is different from those obtained by Pereira da Silva,[26] which showed greater remineralization efficiency in restorations treated with RI when compared to resinous materials without fluoride release. The fluoride presence and its release give to the material a cariostatic effect near restorations margin.[26] So, the difference observed between the results of the studies may be due to the cariogenic challenges time exposure, which is longer (5 days) in the present research.

In the present study, it is observed in the results obtained in the RS, being the material with the greatest gain in hardness, which indicates a greater degree of remineralization or inhibit of demineralization. Regarding the other materials used in this research, resin reinforced glass ionomer also had an anticariogenic effect, however it showed lower results than the silver-reinforced GIC. Composite resin was the material that showed worse results compared to glass ionomer cements, showing higher demineralization in the margins. This better result for the silver-reinforced GIC can be explained by the properties that the silver adds to the metal-reinforced GIC. This addition of silver improves the wear resistance and fragility of GICs,[10] in addition to adding antibacterial properties against gram-positive and gram-negative bacteria.[27,28]

Within the limitations of this single-species biofilm experimental caries model, restorations with GICs appears to interrupt caries lesion progression into enamel. The metabolic activity test assessed only *S. mutans* biofilm, which does not represent all the biofilm present in the oral cavity. Therefore, our results cannot be extended to clinical situations without some restrictions. The demineralization analysis after biofilm induction was carried out using the surface microhardness test, a test that has already proven its effectiveness for this type of study[29] but other methods can be used for the same purpose.

The study suggests the high anti-caries potential of RS treatment, but the need for understanding of mechanism of action for appropriate methods of measurement to be determined will be of crucial importance for the product.

No studies assessing the effect of RS as a secondary carious lesion control agent in enamel specimens have been conducted. Additionally, which of the two types of GIGs is more effective in inhibiting carious lesion development is not clear.

Our results provide evidence that RS is superior to RI and CO as restorative material in preventing recurrent dental caries at distance of 50 µm from the restoration margin. Thus, an important data is that in the absence of fluoride there is a more demineralization in the margin and that materials containing fluoride release and silver in its composition can contribute to the effective control of recurrent caries in children.

CONCLUSION

All glass ionomers increased microhardness of enamel blocks even exposed to cariogenic challenge. Although only the silver reinforced glass ionomer restorations prevented enamel mineral loss at near distance from their margins.

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